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(21) International Application Number: PCT/US98/19805 (22) International Filing Date: 21 September 1998 (21.09.98) (30) Priority Data: 08/934,169 19 September 1997 (19.09.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/934,169 (CIP) Filed on 19 September 1997 (19.09.97) (71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): AU-YOUNG, Janice [US/US]; 1419 Kains Avenue, Berkeley, CA 94702 (US). GUEGLER, Karl, J. [CH/US]; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). CORLEY, Neil, C. [US/US]; 1240 Dale Avenue #30, Mountain View, CA 94040 (US). (74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HUMAN PRL-1 PHOSPHATASE (57) Abstract <p>The invention provides a human PRL-1 phosphatase (HPRL-1) and polynucleotides which identify and encode HPRL-1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of HPRL-1.</p>		

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Human PRL1 Phosphatase

TECHNICAL FIELD

5 This invention relates to nucleic acid and amino acid sequences of a human PRL-1 phosphatase and to the use of these sequences in the diagnosis, prevention, and treatment of diseases associated with cell proliferation.

BACKGROUND OF THE INVENTION

10 Kinases and phosphatases regulate many different cell proliferation, differentiation, and signaling processes by adding and removing phosphate groups to and from proteins. Reversible protein phosphorylation is the main strategy for controlling activities of eukaryotic cells. It is estimated that more than 1000 of the 10,000 proteins active in a typical
15 mammalian cell are phosphorylated. The high energy phosphate which drives activation is generally transferred from adenosine triphosphate molecules (ATP) to a particular protein by protein kinases and removed from that protein by protein phosphatases. Phosphorylation occurs in response to extracellular signals (hormones, neurotransmitters, growth and differentiation factors, etc.), cell cycle checkpoints, and environmental or nutritional stresses and is roughly analogous to turning on a molecular switch. When the switch goes on, the
20 appropriate protein kinase activates a metabolic enzyme, regulatory protein, receptor, cytoskeletal protein, ion channel or pump, or transcription factor. Uncontrolled signaling has been implicated in a variety of disease conditions including inflammation, cancer, arteriosclerosis, and psoriasis.

 Protein tyrosine phosphatases control the extent of phosphorylation of proteins during
25 various cellular signaling events. Reversible phosphorylation of proteins is frequently one step in the action of hormones. The hormone or other physiological effector usually binds to a receptor, which triggers a signaling system that increases or decreases the level of a second messenger such as cyclic AMP, Ca^{2+} ion, or inositol phosphates. The second messenger then activates or inhibits various protein kinases that phosphorylate certain target proteins. The
30 target proteins are sometimes protein phosphatases, which remove the phosphoryl groups. Such a system involves a substantial amplification of the original physiological signal. Nanomolar levels of hormones produce micromolar concentrations of secondary messengers;

each molecule of activated protein kinase or phosphatase catalyzes the phosphorylation or dephosphorylation of many molecules of the target protein.

An example of such a phosphatase is a protein tyrosine phosphatase named PRL-1. PRL-1 like phosphatases may be nuclear or cytosolic and are found in organisms as diverse as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, and rat. Studies have shown PRL-1 is expressed throughout the course of rat hepatic regeneration and its expression is elevated in a number of tumor cell lines. Sequence analysis reveals that the PRL-1 gene encodes a 20-kDa protein with an eight-amino-acid consensus protein tyrosine phosphatase active site. PRL-1 is located primarily in the cell nucleus, is important in normal cellular growth control, and may contribute to the tumorigenicity of some cancer cells (Diamond RH et al. (1994) Mol. Cell Biol. 14(6):3752-3762).

The discovery of a new human PRL-1 phosphatase and the polynucleotides encoding it satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention and treatment of diseases associated with cell proliferation, in particular, cancers and immune responses.

SUMMARY OF THE INVENTION

The invention features a substantially purified polypeptide, human PRL-1 phosphatase (HPRL-1), having the amino acid sequence shown in SEQ ID NO:1, or fragments thereof.

The invention further provides an isolated and substantially purified polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof and a composition comprising said polynucleotide sequence. The invention also provides a polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence encoding the amino acid sequence SEQ ID NO:1, or fragments of said polynucleotide sequence. The invention further provides a polynucleotide sequence comprising the complement of the polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:1, or fragments or variants of said polynucleotide sequence.

The invention also provides an isolated and purified sequence comprising SEQ ID NO:2 or variants thereof. In addition, the invention provides a polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of SEQ ID NO:2. The invention also provides a polynucleotide sequence comprising the complement of SEQ ID NO:2, or fragments or variants thereof.

The present invention further provides an expression vector containing at least a fragment of any of the claimed polynucleotide sequences. In yet another aspect, the expression vector containing the polynucleotide sequence is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment thereof, the method comprising the steps of: a) culturing the host cell containing an expression vector containing at least a fragment of the polynucleotide sequence encoding HPRL-1 under conditions suitable for the expression of the polypeptide; and b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified HPRL-1 having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

The invention also provides a purified antagonist of the polypeptide of SEQ ID NO:1. In one aspect the invention provides a purified antibody which binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:1.

Still further, the invention provides a purified agonist of the polypeptide of SEQ ID NO:1.

The invention also provides a method for treating or preventing an immune response comprising administering to a subject in need of such treatment an effective amount of an antagonist to HPRL-1.

The invention also provides a method for treating a cancer comprising administering to a subject in need of such treatment an effective amount of an antagonist to HPRL-1.

The invention also provides a method for detecting a polynucleotide which encodes HPRL-1 in a biological sample comprising the steps of: a) hybridizing the complement of the polynucleotide sequence which encodes SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and b) detecting the hybridization complex, wherein the presence of the complex correlates with the presence of a polynucleotide encoding HPRL-1 in the biological sample. In one aspect the nucleic acid material of the biological sample is amplified by the polymerase chain reaction prior to hybridization.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, 1C, 1D, 1E, and 1F show the amino acid sequence (SEQ ID NO:1)

and nucleic acid sequence (SEQ ID NO:2) of HPRL-1. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd. San Bruno, CA).

Figures 2A and 2B show the amino acid sequence alignments among HPRL-1 (78563; SEQ ID NO:1), and Arabidopsis thaliana HPRL-1 (GI 577733; SEQ ID NO:3), produced
5 using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

Figures 3A and 3B show the hydrophobicity plots for HPRL-1 (SEQ ID NO:1) and Arabidopsis thaliana PRL-1 gene product (SEQ ID NO:3), respectively; the positive X axis reflects amino acid position, and the negative Y axis, hydrophobicity (MacDNASIS PRO
10 software).

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell
15 lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms
20 “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a host cell” includes a plurality of such host cells, reference to the “antibody” is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same
25 meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and
30 methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

HPRL-1, as used herein, refers to the amino acid sequences of substantially purified HPRL-1 obtained from any species, particularly mammalian, including bovine, ovine,
5 porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist", as used herein, refers to a molecule which, when bound to HPRL-1, increases or prolongs the duration of the effect of HPRL-1. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of
10 HPRL-1.

An "allele" or "allelic sequence", as used herein, is an alternative form of the gene encoding HPRL-1. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic
15 forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HPRL-1 as used herein include those with deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that
20 encodes the same or a functionally equivalent HPRL-1. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HPRL-1, and improper or unexpected hybridization to alleles, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HPRL-1. The encoded protein may also be "altered" and contain
25 deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HPRL-1. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological or immunological activity of HPRL-1 is retained. For example, negatively charged amino acids may include
30 aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine, glycine and alanine, asparagine and

glutamine, serine and threonine, and phenylalanine and tyrosine.

“Amino acid sequence” as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragment thereof, and to naturally occurring or synthetic molecules. Fragments of HPRL-1 are preferably about 5 to about 15 amino acids in length and retain the biological activity or the immunological activity of HPRL-1. Where “amino acid sequence” is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, amino acid sequence, and like terms, are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

“Amplification” as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY).

The term “antagonist” as used herein, refers to a molecule which, when bound to HPRL-1, decreases the amount or the duration of the effect of the biological or immunological activity of HPRL-1. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies or any other molecules which decrease the effect of HPRL-1.

As used herein, the term “antibody” refers to intact molecules as well as fragments thereof, such as Fa, F(ab')₂, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind HPRL-1 polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal can be derived from the translation of RNA or synthesized chemically and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin, keyhole limpet hemocyanin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term “antigenic determinant”, as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense", as used herein, refers to any composition containing nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules include peptide nucleic acids and may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and block either transcription or translation. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HPRL-1, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands and in the design and use of PNA molecules.

A "composition comprising a given polynucleotide sequence" as used herein refers broadly to any composition containing the given polynucleotide sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HPRL-1 (SEQ ID NO:1) or fragments thereof (e.g., SEQ ID NO:2 and fragments thereof) may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., SDS) and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

“Consensus”, as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly (e.g., GELVIEW™ Fragment Assembly system, GCG, Madison, WI).
Some sequences have been both extended and assembled to produce the consensus sequence .

The term “correlates with expression of a polynucleotide”, as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 by northern analysis is indicative of the presence of mRNA encoding HPRL-1 in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

A “deletion”, as used herein, refers to a change in the amino acid or nucleotide sequence and results in the absence of one or more amino acid residues or nucleotides.

The term “derivative”, as used herein, refers to the chemical modification of a nucleic acid encoding or complementary to HPRL-1 or the encoded HPRL-1. Such modifications include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative encodes a polypeptide which retains the biological or immunological function of the natural molecule. A derivative polypeptide is one which is modified by glycosylation, pegylation, or any similar process which retains the biological or immunological function of the polypeptide from which it was derived.

The term “homology”, as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to using the functional term “substantially homologous.” The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or hybridization probe will compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a

second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity). In the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

Human artificial chromosomes (HACs) are linear microchromosomes which may contain DNA sequences of 10K to 10M in size and contain all of the elements required for stable mitotic chromosome segregation and maintenance (Harrington, J.J. et al. (1997) Nat Genet. 15:345-355).

The term "humanized antibody", as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability.

The term "hybridization", as used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex", as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, as compared to the naturally occurring molecule.

"Microarray" refers to an array of distinct polynucleotides or oligonucleotides synthesized on a substrate, such as paper, nylon or other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The term "modulate", as used herein, refers to a change in the activity of HPRL-1. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional or immunological properties of HPRL-1.

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments thereof, and to DNA or RNA of genomic or synthetic origin

which may be single- or double-stranded, and represent the sense or antisense strand. "Fragments" are those nucleic acid sequences which are greater than 60 nucleotides in length, and most preferably includes fragments that are at least 100 nucleotides or at least 1000 nucleotides, and at least 10,000 nucleotides in length.

5 The term "oligonucleotide" refers to a nucleic acid sequence of at least about 6 nucleotides to about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20 to 25 nucleotides, which can be used in PCR amplification or a hybridization assay, or a microarray. As used herein, oligonucleotide is substantially equivalent to the terms "amplimers", "primers", "oligomers", and "probes", as commonly
10 defined in the art.

 "Peptide nucleic acid", PNA as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues which ends in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan
15 in the cell where they preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63).

 The term "portion", as used herein, with regard to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from five amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein
20 "comprising at least a portion of the amino acid sequence of SEQ ID NO:1" encompasses the full-length HPRL-1 and fragments thereof.

 The term "sample", as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding HPRL-1, or fragments thereof, or HPRL-1 itself may comprise a bodily fluid, extract from a cell, chromosome, organelle, or membrane
25 isolated from a cell, a cell, genomic DNA, RNA, or cDNA (in solution or bound to a solid support, a tissue, a tissue print, and the like).

 The terms "specific binding" or "specifically binding", as used herein, refers to that interaction between a protein or peptide and an agonist, an antibody and an antagonist. The interaction is dependent upon the presence of a particular structure (i.e., the antigenic
30 determinant or epitope) of the protein recognized by the binding molecule. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of

labeled A bound to the antibody.

The terms "stringent conditions" or "stringency", as used herein, refer to the conditions for hybridization as defined by the nucleic acid, salt, and temperature. These conditions are well known in the art and may be altered in order to identify or detect identical or related polynucleotide sequences. Numerous equivalent conditions comprising either low or high stringency depend on factors such as the length and nature of the sequence (DNA, RNA, base composition), nature of the target (DNA, RNA, base composition), milieu (in solution or immobilized on a solid substrate), concentration of salts and other components (e.g., formamide, dextran sulfate and/or polyethylene glycol), and temperature of the reactions (within a range from about 5°C below the melting temperature of the probe to about 20°C to 25°C below the melting temperature). One or more factors may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation", as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

A "variant" of HPRL-1, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of

leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

THE INVENTION

The invention is based on the discovery of a new human PRL-1 phosphatase (hereinafter referred to as "HPRL-1"), the polynucleotides encoding HPRL-1, and the use of these compositions for the diagnosis, prevention, or treatment of cancer and immune response.

Nucleic acids encoding the HPRL-1 of the present invention were first identified in Incyte Clone 78563 from the rheumatoid synovium cDNA library (SYNORAB01) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 78563 (SYNORAB01), 2632064 (COLNTUT15), 155679 (THP1PLB02), 81202 (SYNORAB01), and 1710173 (PROSNOT16).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figs. 1A, 1B, 1C, 1D, 1E, and 1F. HPRL-1 is 457 amino acids in length and has five potential casein kinase II phosphorylation sites at residues T₆₀, T₁₃₄, S₁₄₄, S₂₆₃, and S₄₂₉; twelve potential protein kinase C phosphorylation sites at residues S₁₇, S₃₉, T₆₀, S₁₅₄, T₂₂₇, S₂₃₅, T₂₄₈, S₂₅₅, T₃₁₁, T₃₃₅, T₃₅₃, and S₃₇₄; one potential tyrosine kinase phosphorylation site at residue Y₂₇₇; and two beta-transducin family Trp-Asp repeats signature sites at residues L₃₀₃ to V₃₁₇ and I₃₄₅ to L₃₅₉. As shown in Figs. 2A and 2B, HPRL-1 has chemical and structural homology with *Arabidopsis thaliana* (GI 577733; SEQ ID NO:3). In particular, HPRL-1 and *Arabidopsis thaliana* PRL-1 share 40% identity and the casein kinase II phosphorylation site at residue S₄₂₉, the protein kinase C phosphorylation sites at S₁₇, S₁₅₄, T₂₂₇, S₂₅₅, T₃₅₃, and S₄₃₇, and both of the beta-transducin family Trp-Asp sites. As illustrated by Figs. 3A and 3B, HPRL-1 and *Arabidopsis thaliana* PRL-1 gene product have rather similar hydrophobicity plots. Northern analysis shows the expression of HPRL-1 in various cDNA libraries, at least 40% of which are immortalized or cancerous, at least 34% of

which involve immune response, and at least 20% of which involve fetal/infant development.

The invention also encompasses HPRL-1 variants. A preferred HPRL-1 variant is one having at least 80%, and more preferably at least 90%, amino acid sequence identity to the HPRL-1 amino acid sequence (SEQ ID NO:1) and which retains at least one biological, immunological or other functional characteristic or activity of HPRL-1. A most preferred HPRL-1 variant is one having at least 95% amino acid sequence identity to SEQ ID NO:1.

The invention also encompasses polynucleotides which encode HPRL-1. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of HPRL-1 can be used to produce recombinant molecules which express HPRL-1. In a particular embodiment, the invention encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figs. 1A, 1B, 1C, 1D, 1E, and 1F.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding HPRL-1, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HPRL-1, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HPRL-1 and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HPRL-1 under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HPRL-1 or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HPRL-1 and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences, or fragments thereof, which encode HPRL-1 and its derivatives, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and

cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HPRL-1 or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2, under various conditions of stringency as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511).

Methods for DNA sequencing which are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE Amplification System marketed by Gibco/BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

The nucleic acid sequences encoding HPRL-1 may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using commercially available software such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target

sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR
5 amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

10 Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been
15 size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

20 Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light
25 intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

30 In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HPRL-1 may be used in recombinant DNA molecules to direct expression of HPRL-1, fragments or functional equivalents thereof, in appropriate host cells.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced, and these sequences may be used to clone and express HPRL-1.

As will be understood by those of skill in the art, it may be advantageous to produce
5 HPRL-1-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

10 The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HPRL-1 encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the
15 nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HPRL-1 may be ligated to a heterologous sequence to encode a
20 fusion protein. For example, to screen peptide libraries for inhibitors of HPRL-1 activity, it may be useful to encode a chimeric HPRL-1 protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the HPRL-1 encoding sequence and the heterologous protein sequence, so that HPRL-1 may be cleaved and purified away from the heterologous moiety.

25 In another embodiment, sequences encoding HPRL-1 may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of HPRL-1, or a fragment thereof. For example, peptide synthesis can
30 be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence of HPRL-1, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active HPRL-1, the nucleotide sequences encoding HPRL-1 or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HPRL-1 and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HPRL-1. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible

promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the
5 genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding HPRL-1, vectors based on SV40 or EBV may be used with an appropriate
10 selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for HPRL-1. For example, when large quantities of HPRL-1 are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the
15 multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding HPRL-1 may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to
20 express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released
25 from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences
30 encoding HPRL-1 may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311).

Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express HPRL-1. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding HPRL-1 may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of HPRL-1 will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which HPRL-1 may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HPRL-1 may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing HPRL-1 in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6 to 10M are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HPRL-1. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding HPRL-1, its initiation codon, and upstream

sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the
5 correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

10 In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding
15 and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is
20 preferred. For example, cell lines which stably express HPRL-1 may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable
25 marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines.
30 These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk⁻ or aprt⁻ cells, respectively. Also,

antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, β glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding HPRL-1 is inserted within a marker gene sequence, transformed cells containing sequences encoding HPRL-1 can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HPRL-1 under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding HPRL-1 and express HPRL-1 may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of polynucleotide sequences encoding HPRL-1 can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding HPRL-1. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding HPRL-1 to detect transformants containing DNA or RNA encoding HPRL-1.

A variety of protocols for detecting and measuring the expression of HPRL-1, using

either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HPRL-1 is
5 preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the
10 art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HPRL-1 include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HPRL-1, or any fragments thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are
15 known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH). Suitable reporter molecules or labels, which may be used for ease of
20 detection, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HPRL-1 may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or contained intracellularly
25 depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HPRL-1 may be designed to contain signal sequences which direct secretion of HPRL-1 through a prokaryotic or eukaryotic cell membrane. Other constructions may be used to join sequences encoding HPRL-1 to nucleotide sequence encoding a polypeptide domain which will facilitate
30 purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on

immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and HPRL-1 may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing HPRL-1 and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3: 263-281) while the enterokinase cleavage site provides a means for purifying HPRL-1 from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

In addition to recombinant production, fragments of HPRL-1 may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of HPRL-1 may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

THERAPEUTICS

Chemical and structural homology exists between HPRL-1 and PLR-1 gene product from Arabidopsis thaliana (GI 577733). Northern analysis shows that the expression of HPRL-1 is associated with cell proliferation, cancer, and immune response.

Therefore, in one embodiment, an antagonist of HPRL-1 may be administered to a subject to prevent or treat an immune response of any type and, in particular, that which is associated with a particular disorder.. Such disorders associated with an immune response include, but are not limited to AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid

arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma. In one aspect, an antibody which specifically binds HPRL-1 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HPRL-1.

In another embodiment, a vector expressing the complement of the polynucleotide encoding HPRL-1 may be administered to a subject to treat or prevent an immune response as associated with, but not limited to, the disorders described above.

In another embodiment, an antagonist of HPRL-1 or a fragment or derivative thereof may be administered to a subject to treat or prevent a cancer. Such cancers include, but are not limited to, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma; and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. In one aspect, an antibody which specifically binds HPRL-1 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HPRL-1.

In another embodiment, a vector capable of expressing the complement of the polynucleotide encoding HPRL-1, may be administered to a subject to treat or prevent a cancer including, but not limited to, those cancers listed above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HPRL-1 may be produced using methods which are generally known in the art. In particular, purified HPRL-1 may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HPRL-1.

Antibodies to HPRL-1 may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with HPRL-1 or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HPRL-1 have an amino acid sequence consisting of at least five amino acids and more preferably at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HPRL-1 amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Monoclonal antibodies to HPRL-1 may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known

in the art, to produce HPRL-1-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-3).

5 Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

10 Antibody fragments which contain specific binding sites for HPRL-1 may also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W.D. et al. 15 (1989) Science 254:1275-1281).

 Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex 20 formation between HPRL-1 and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HPRL-1 epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

 In another embodiment of the invention, the polynucleotides encoding HPRL-1, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, 25 the complement of the polynucleotide encoding HPRL-1 may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HPRL-1. Thus, complementary molecules or fragments may be used to modulate HPRL-1 activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense 30 or antisense oligonucleotides or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding HPRL-1.

 Expression vectors derived from retro viruses, adenovirus, herpes or vaccinia viruses,

or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequence which is complementary to the polynucleotides of the gene encoding HPRL-1. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra).

Genes encoding HPRL-1 can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes HPRL-1. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions of the gene encoding HPRL-1 (signal sequence, promoters, enhancers, and introns). Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples which may be used include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HPRL-1.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the

following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by
5 testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase
10 phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HPRL-1. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

15 RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of
20 nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced
25 into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections or polycationic amino polymers (Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-66; incorporated herein by reference) may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any subject in need
30 of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a

pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HPRL-1, antibodies to HPRL-1, mimetics, agonists, antagonists, or inhibitors of HPRL-1. The compositions may be administered alone or in combination with at least one
5 other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by
10 any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which
15 facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using
20 pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of
25 active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium
30 carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium

alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the

corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HPRL-1, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HPRL-1 or fragments thereof, antibodies of HPRL-1, agonists, antagonists or inhibitors of HPRL-1, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age,

weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

5 Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be
10 specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HPRL-1 may be used for the diagnosis of conditions or diseases characterized by expression of HPRL-1, or in assays to
15 monitor patients being treated with HPRL-1, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for HPRL-1 include methods which utilize the antibody and a label to detect HPRL-1 in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by
20 joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring HPRL-1 are known in the art and provide a basis for diagnosing altered or abnormal levels of HPRL-1
25 expression. Normal or standard values for HPRL-1 expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to HPRL-1 under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric means. Quantities of HPRL-1 expressed in subject, control and disease, samples
30 from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HPRL-1 may

be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of HPRL-1 may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of HPRL-1, and to monitor regulation of HPRL-1 levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HPRL-1 or closely related molecules, may be used to identify nucleic acid sequences which encode HPRL-1. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding HPRL-1, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the HPRL-1 encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:2 or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring HPRL-1.

Means for producing specific hybridization probes for DNAs encoding HPRL-1 include the cloning of nucleic acid sequences encoding HPRL-1 or HPRL-1 derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as ³²P or ³⁵S, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HPRL-1 may be used for the diagnosis of conditions or disorders which are associated with expression of HPRL-1. Examples of such conditions or disorders include adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma; and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney,

liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and immune responses associated with disorders such as AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma. The polynucleotide sequences encoding HPRL-1 may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dipstick, pin, ELISA assays or microarrays utilizing fluids or tissues from patient biopsies to detect altered HPRL-1 expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HPRL-1 may be useful in assays that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding HPRL-1 may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding HPRL-1 in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of disease associated with expression of HPRL-1, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes HPRL-1, under conditions suitable for hybridization or amplification. Standard hybridization may be

quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HPRL-1 may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'→3') and another with antisense (3'←5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of HPRL-1 include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J. Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, an oligonucleotide derived from any of the polynucleotide

sequences described herein may be used as a target in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously (to produce a transcript image), and to identify genetic variants, mutations and polymorphisms. This information will be useful in determining gene function, understanding the genetic basis of disease, diagnosing disease, and in developing and monitoring the activity of therapeutic agents (Heller, R. et al. (1997) *Proc. Natl. Acad. Sci.* 94:2150-55) .

In one embodiment, the microarray is prepared and used according to the methods described in PCT application WO95/11995 (Chee et al.), Lockhart, D. J. et al. (1996; *Nat. Biotech.* 14: 1675-1680) and Schena, M. et al. (1996; *Proc. Natl. Acad. Sci.* 93: 10614-10619), all of which are incorporated herein in their entirety by reference.

The microarray is preferably composed of a large number of unique, single-stranded nucleic acid sequences, usually either synthetic antisense oligonucleotides or fragments of cDNAs, fixed to a solid support. The oligonucleotides are preferably about 6-60 nucleotides in length, more preferably 15-30 nucleotides in length, and most preferably about 20-25 nucleotides in length. For a certain type of microarray, it may be preferable to use oligonucleotides which are only 7-10 nucleotides in length. The microarray may contain oligonucleotides which cover the known 5', or 3', sequence, sequential oligonucleotides which cover the full length sequence; or unique oligonucleotides selected from particular areas along the length of the sequence. Polynucleotides used in the microarray may be oligonucleotides that are specific to a gene or genes of interest in which at least a fragment of the sequence is known or that are specific to one or more unidentified cDNAs which are common to a particular cell type, developmental or disease state.

In order to produce oligonucleotides to a known sequence for a microarray, the gene of interest is examined using a computer algorithm which starts at the 5' or more preferably at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack predicted secondary structure that may interfere with hybridization. In certain situations it may be appropriate to use pairs of oligonucleotides on a microarray. The "pairs" will be identical, except for one nucleotide which preferably is located in the center of the sequence. The second oligonucleotide in the pair (mismatched by one) serves as a control. The number of oligonucleotide pairs may range from two to one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be

paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support.

In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application WO95/251116 (Baldeschweiler et al.) which is incorporated herein in its entirety by reference. In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot blot apparatus), materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and one million which lends itself to the efficient use of commercially available instrumentation.

In order to conduct sample analysis using a microarray, the RNA or DNA from a biological sample is made into hybridization probes. The mRNA is isolated, and cDNA is produced and used as a template to make antisense RNA (aRNA). The aRNA is amplified in the presence of fluorescent nucleotides, and labeled probes are incubated with the microarray so that the probe sequences hybridize to complementary oligonucleotides of the microarray. Incubation conditions are adjusted so that hybridization occurs with precise complementary matches or with various degrees of less complementarity. After removal of nonhybridized probes, a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to determine degree of complementarity and the relative abundance of each oligonucleotide sequence on the microarray. The biological samples may be obtained from any bodily fluids (such as blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. A detection system may be used to measure the absence, presence, and amount of hybridization for all of the distinct sequences simultaneously. This data may be used for large scale correlation studies on the sequences, mutations, variants, or polymorphisms among samples.

In another embodiment of the invention, the nucleic acid sequences which encode HPRL-1 may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome or to artificial chromosome constructions,

such as human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) *Blood Rev.* 7:127-134, and Trask, B.J. (1991) *Trends Genet.* 7:149-154.

5 Fluorescent in situ hybridization (FISH as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in various scientific journals or at Online Mendelian Inheritance in Man (OMIM). Correlation between the location of the gene
10 encoding HPRL-1 on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques
15 such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable
20 information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) *Nature* 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention
25 may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

 In another embodiment of the invention, HPRL-1, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free
30 in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between HPRL-1 and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to HPRL-1 large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with HPRL-1, or fragments thereof, and washed. Bound HPRL-1 is then detected by methods well known in the art. Purified HPRL-1 can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HPRL-1 specifically compete with a test compound for binding HPRL-1. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HPRL-1.

In additional embodiments, the nucleotide sequences which encode HPRL-1 may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

EXAMPLES

I SYNORAB01 cDNA Library Construction

The SYNORAB01 cDNA library was constructed from total RNA from the synovium of a rheumatoid elbow. The rheumatoid synovial tissue was obtained from UC Davis (lot #48) where it had been removed from a 51 year old Asian female and frozen. The frozen tissue was ground in a mortar and pestle and lysed immediately in a buffer containing guanidinium isothiocyanate. The lysate was extracted twice with phenol chloroform at pH 8.0 and centrifuged over a CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol and resuspended in water.

The RNA for SYNORAB01 which was used in the SuperScript Plasmid System (catalogue #18248-013; Gibco/BRL, Gaithersburg MD) with the recommended protocol.

cDNAs were fractionated on a Sepharose CL4B column (catalog #275105, Pharmacia), and those cDNAs exceeding 1 kb were ligated into pSport I. The plasmid was transformed into chemically competent DH5 α host cells (Gibco/BRL).

5 II Isolation and Sequencing of cDNA Clones

Plasmid DNA was purified using the Miniprep Kit (Catalogue # 77468, GIBCO/BRL), a 96-well block kit with reagents for 960 purifications. The recommended protocol included with the kit was employed except for the following changes. Each of the 96 wells was filled with only 1 ml of sterile Terrific Broth (Catalog # 22711, GIBCO/BRL) with carbenicillin at
10 25 mg/L and glycerol at 0.4%. After the wells were inoculated, the bacteria were cultured for 24 hours and lysed with 60 μ l of lysis buffer. A centrifugation step (Beckman GS-6R @2900 rpm for 5 min; Beckman Instruments) was performed before the contents of the block were added to the primary filter plate. The optional step of adding isopropanol to TRIS buffer was not routinely performed. After the last step in the protocol, samples were transferred to a
15 Beckman 96-well block for storage.

The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer) and reading frame was
20 determined.

III Homology Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences of the Sequence Listing or amino acid sequences deduced from them were used as query sequences against databases such as GenBank, SwissProt,
25 BLOCKS, and Pima II. These databases which contain previously identified and annotated sequences were searched for regions of homology (similarity) using BLAST, which stands for Basic Local Alignment Search Tool (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10).

BLAST produces alignments of both nucleotide and amino acid sequences to
30 determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal or plant) origin. Other algorithms such as the one described in Smith RF and TF Smith (1992 Protein Engineering 5:35-51), incorporated

herein by reference, can be used when dealing with primary sequence patterns and secondary structure gap penalties. As disclosed in this application, the minimum length of the sequences in the Sequence Listing is 49 nucleotides, and the upper limit of uncalled bases where N is recorded rather than A, C, G, or T is 12%.

5 The BLAST approach, as detailed in Karlin and Altschul (1993; Proc Nat Acad Sci 90:5873-7) and incorporated herein by reference, searches matches between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. In this application, threshold was set at 10-25 for nucleotides and 10-14 for peptides.

10 Incyte nucleotide sequence were searched against the GenBank databases for pri=primate, rod=rodent, and mam=mammalian sequences, and deduced amino acid sequences from the same clones are searched against GenBank functional protein databases, mamp=mammalian, vrtp=vertebrate and eukp=eukaryote, for homology.

15 IV Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., supra).

Analogous computer techniques using BLAST (Altschul, S.F. (1993) J.Mol.Evol. 36:290-300; Altschul, S.F. et al. (1990) J.Mol.Evol. 215:403-410) are used to search for 20 identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

25 The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be 30 exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding HPRL-1 occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V Extension of HPRL-1 Encoding Polynucleotides

The nucleic acid sequence of the Incyte Clone 78563 was used to design oligonucleotide primers for extending a partial nucleotide sequence to full length. One primer was synthesized to initiate extension in the antisense direction, and the other was synthesized to extend sequence in the sense direction. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be about 22 to about 30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures of about 68° to about 72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (Gibco/BRL) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR was performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles

Step 12 72° C for 8 min
 Step 13 4° C (and holding)

A 5-10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a low
 5 concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful
 in extending the sequence. Bands thought to contain the largest products were excised from
 the gel, purified using QIAQuick™ (QIAGEN Inc., Chatsworth, CA), and trimmed of
 overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μ l of ligation buffer,
 10 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase were added, and the mixture
 was incubated at room temperature for 2-3 hours or overnight at 16° C. Competent E. coli
 cells (in 40 μ l of appropriate media) were transformed with 3 μ l of ligation mixture and
 cultured in 80 μ l of SOC medium (Sambrook et al., supra). After incubation for one hour at
 37° C, the E. coli mixture was plated on Luria Bertani (LB)-agar (Sambrook et al., supra)
 15 containing 2x Carb. The following day, several colonies were randomly picked from each
 plate and cultured in 150 μ l of liquid LB/2x Carb medium placed in an individual well of an
 appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μ l
 of each overnight culture was transferred into a non-sterile 96-well plate and after dilution
 1:10 with water, 5 μ l of each sample was transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4
 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers
 used for the extension reaction were added to each well. Amplification was performed using
 the following conditions:

Step 1 94° C for 60 sec
 Step 2 94° C for 20 sec
 Step 3 55° C for 30 sec
 Step 4 72° C for 90 sec
 Step 5 Repeat steps 2-4 for an additional 29 cycles
 Step 6 72° C for 180 sec
 Step 7 4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular
 weight markers. The sizes of the PCR products were compared to the original partial cDNAs,
 and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of SEQ ID NO:2 is used to obtain 5'
 regulatory sequences using the procedure above, oligonucleotides designed for 5' extension,

and an appropriate genomic library.

VI Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs,
5 genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20
base-pairs, is specifically described, essentially the same procedure is used with larger
nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as
OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250
10 μ Ci of [γ - 32 P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont
NEN[®], Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex
G-25 superfine resin column (Pharmacia & Upjohn). A aliquot containing 10^7 counts per
minute of the labeled probe is used in a typical membrane-based hybridization analysis of
human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI,
Pst I, Xba 1, or Pvu II; DuPont NEN[®]).

15 The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred
to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is
carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed
at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate
and 0.5% sodium dodecyl sulfate. After XOMAT AR[™] film (Kodak, Rochester, NY) is
20 exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale, CA) for
several hours, hybridization patterns are compared visually.

VII Microarrays

To produce oligonucleotides for a microarray, the nucleotide sequence described
25 herein is examined using a computer algorithm which starts at the 3' end of the nucleotide
sequence. The algorithm identifies oligomers of defined length that are unique to the gene,
have a GC content within a range suitable for hybridization, and lack predicted secondary
structure that would interfere with hybridization. The algorithm identifies 20 sequence-
specific oligonucleotides of 20 nucleotides in length (20-mers). A matched set of
30 oligonucleotides is created in which one nucleotide in the center of each sequence is altered.
This process is repeated for each gene in the microarray, and double sets of twenty 20 mers are
synthesized and arranged on the surface of the silicon chip using a light-directed chemical

process (Chee, M. et al., PCT/WO95/11995, incorporated herein by reference).

In the alternative, a chemical coupling procedure and an ink jet device are used to synthesize oligomers on the surface of a substrate (Baldeschweiler, J.D. et al., PCT/WO95/25116, incorporated herein by reference). In another alternative, a "gridded" array
5 analogous to a dot (or slot) blot is used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array may be produced by hand or using available materials and machines and contain grids of 8 dots, 24 dots, 96 dots, 384 dots, 1536 dots or 6144 dots. After hybridization, the microarray is washed to remove nonhybridized probes, and
10 a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to determine degree of complementarity and the relative abundance of each oligonucleotide sequence on the micro-array.

VIII Complementary Polynucleotides

15 Sequence complementary to the HPRL-1-encoding sequence, or any part thereof, is used to decrease or inhibit expression of naturally occurring HPRL-1. Although use of oligonucleotides comprising from about 15 to about 30 base-pairs is described, essentially the same procedure is used with smaller or larger sequence fragments. Appropriate oligonucleotides are designed using Oligo 4.06 software and the coding sequence of HPRL-1,
20 SEQ ID NO:1. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HPRL-1-encoding transcript.

25 IX Expression of HPRL-1

Expression of HPRL-1 is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector is also used to express HPRL-1 in *E. coli*. Upstream of the cloning site, this vector contains a promoter for β -galactosidase, followed by sequence containing the amino-terminal Met, and
30 the subsequent seven residues of β -galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of β -galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of HPRL-1 into the bacterial growth media which can be used directly in the following assay for activity.

X Demonstration of HPRL-1 Activity

The enzymatic assay is performed in 100 mM sodium acetate, pH 5.0, 1 mM EDTA at the ionic strength of 0.15 M which is adjusted using sodium chloride. The rate of dephosphorylation activity of HPRL-1 is established by measuring the release of inorganic phosphate using a malachite green assay procedure or by monitoring the liberation of *p*-nitrophenol at 405 nm absorbance as described by (Ostanin K. et al. (1995) J. Biol. Chem. 270(31):18491-18499).

XI Production of HPRL-1 Specific Antibodies

HPRL-1 that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols. The amino acid sequence deduced from SEQ ID NO:2 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel et al. (supra), and others.

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio iodinated, goat anti-rabbit IgG.

XII Purification of Naturally Occurring HPRL-1 Using Specific Antibodies

Naturally occurring or recombinant HPRL-1 is substantially purified by

immunoaffinity chromatography using antibodies specific for HPRL-1. An immunoaffinity column is constructed by covalently coupling HPRL-1 antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HPRL-1 is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HPRL-1 (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HPRL-1 binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HPRL-1 is collected.

XIII Identification of Molecules Which Interact with HPRL-1

HPRL-1 or biologically active fragments thereof are labeled with ^{125}I Bolton-Hunter reagent (Bolton et al. (1973) Biochem. J. 133: 529). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HPRL-1, washed and any wells with labeled HPRL-1 complex are assayed. Data obtained using different concentrations of HPRL-1 are used to calculate values for the number, affinity, and association of HPRL-1 with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

What is claimed is:

1. A substantially purified human PRL-1 phosphatase comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.

5

2. A substantially purified variant of human PRL-1 phosphatase having at least 90% amino acid identity to SEQ ID NO:1 and which retains at least one functional characteristic of PRL-1 gene product.

10 3. An isolated and purified polynucleotide sequence encoding the human PRL-1 phosphatase of claim 1 or fragments or variants of said polynucleotide sequence.

4. A composition comprising the polynucleotide sequence of claim 3.

15 5. A polynucleotide sequence which hybridizes to the polynucleotide sequence of claim 3.

6. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 3 or fragments or variants thereof.

20

7. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or fragments or variants thereof.

25 8. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 7.

9. An expression vector containing at least a fragment of the polynucleotide sequence of claim 3.

30 10. A host cell containing the vector of claim 9.

11. A method for producing a polypeptide comprising the amino acid sequence of

SEQ ID NO:1, or a fragment thereof, the method comprising the steps of:

- a) culturing the host cell of claim 10 under conditions suitable for the expression of the polypeptide; and
- b) recovering the polypeptide from the host cell culture.

5

12. A pharmaceutical composition comprising a substantially purified human PRL-1 phosphatase having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

10 13. A purified antibody which specifically binds to the polypeptide of claim 1.

14. A purified agonist of the polypeptide of claim 1.

15. A purified antagonist of the polypeptide of claim 1.

15

16. A method for treating an immune response comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 15.

17. A method for treating a cancer comprising administering to a subject in need of
20 such treatment an effective amount of the antagonist of claim 15.

18. A method for detecting a polynucleotide which encodes human PRL-1 phosphatase in a biological sample comprising the steps of:

- a) hybridizing the polynucleotide of claim 6 to nucleic acid material of a
25 biological sample, thereby forming a hybridization complex; and
- b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding human PRL-1 phosphatase in said biological sample.

19. The method of claim 18 wherein the nucleic acid material is amplified by the
30 polymerase chain reaction prior to hybridization.

1/10

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9      18      27      36      45      54
NNG GGC GCC CAA TTC CGG AAG GTG CTG CAC AGC TGT GGC GGC TAC TGC GTT

63     72     81     90     99    108
AGT GAT TAG AGT TTC TTC CCT GCC GGA GGT GGG ATA CAC GGT AGC ATC ATG GTC
      M      V

117    126    135    144    153    162
GAG GAG GTA CAG AAA CAT TCT GTA CAC ACC CTT GTG TTC AGG TCG TTG AAG AGG
E  E  V  Q  K  H  S  V  H  T  L  V  F  R  S  L  K  R

171    180    189    198    207    216
ACC CAT GAC ATG TTT GTA GCT GAT AAT GGA AAA CCT GTG CCT TTA GAT GAA GAG
T  H  D  M  F  V  A  D  N  G  K  P  V  P  L  D  E  E

225    234    243    252    261    270
AGT CAC AAA CGA AAA ATG GCA ATC AAG CTT CGT AAT GAG TAT GGT CCT GTG TTG
S  H  K  R  K  M  A  I  K  L  R  N  E  Y  G  P  V  L

279    288    297    306    315    324
CAT ATG CCT ACT TCA AAA GAA AAT CTT AAA GAG AAG GGT CCT CAG AAT GCA ACG
H  M  P  T  S  K  E  N  L  K  E  K  G  P  Q  N  A  T

333    342    351    360    369    378
GAT TCA TAT GTT CAT AAA CAG TAC CCT GCC AAT CAA GGA CAA GAA GTT GAA TAC
D  S  Y  V  H  K  Q  Y  P  A  N  Q  G  Q  E  V  E  Y

```

FIGURE 1A

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387	396	405	414	423	432
TTT GTG GCA GGT ACA CAT CCA TAC CCA GGA CCT GGT GGT TTTG ACA GCA					
F V A G T H P Y P P G P G V A L T A					
441	450	459	468	477	486
GAT ACT AAG ATC CAG AGA ATG CCA AGT GAA TCA GCT GCA CAG TCC TTA GCG GTG					
D T K I Q R M P S E S A A Q S L A V					
495	504	513	522	531	540
GCA TTA CCT TTG CAG ACC AAG GCT GAT GCA AAT CGT ACT GCC CCT AGT GGA AGT					
A L P L Q T K A D A N R T A P S G S					
549	558	567	576	585	594
GAA TAC CGA CAT CCT GGG GCT TCT GAC CGT CCA CAG CCT ACA GCG ATG AAT TCA					
E Y R H P G A S D R P Q P T A M N S					
603	612	621	630	639	648
ATT GTC ATG GAG ACT GGC AAT ACC AAG AAC TCT GCA CTG ATG GCT AAA AAA GCC					
I V M E T G N T K N S A L M A K K A					
657	666	675	684	693	702
CCT ACA ATG CCA AAA CCC CAG TGG CAC CCA CCG TGG AAA CTC TAC AGG GTT ATC					
P T M P K P Q W H P P W K L Y R V I					

FIGURE 1B

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711	AGT GGG CAT CTT GGC TGG GTT CGA TGT ATT GCT GTG GAA CCT GGA AAT CAG TGG	720	729	738	747	756
S G H L G W V R C I A V E P G N Q W						
765	TTT GTT ACT GGA TCT GCT GAC AGA ACT ATA AAG ATC TGG GAC TTG GCT AGT GGC	774	783	792	801	810
F V T G S A D R T I K I W D L A S G						
819	AAA TTA AAA CTG TCA TTG ACT GGG CAT ATT AGT ACT GTG CGG GTG ATA GTA	828	837	846	855	864
K L K L S L T G H I S T V R G V I V						
873	AGC ACA AGG AGC CCA TAT CTG TTC TCT TGT GGA GAA GAC AAA CAA GTG AAA TGC	882	891	900	909	918
S T R S P Y L F S C G E D K Q V K C						
927	TGG GAT CTC GAA TAC AAT AAG GTT ATA CGG CAT TAT CAT GGA CAT TTA AGT GCA	936	945	954	963	972
W D L E Y N K V I R H Y H G H L S A						
981	GTG TAT GGT TTG GAT TTG CAC CCG ACA ATC GAT GTG TTG GTA ACC TGT AGT CGA	990	999	1008	1017	1026
V Y G L D L H P T I D V L V T C S R						

FIGURE 1C

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1035	1044	1053	1062	1071	1080
GAT TCA ACT GCA CGG ATT TGG GAT GTG AGA ACT AAA GCC AGT GTA CAC ACA TTA					
D S T A R I W D V R T K A S V H T L					
1089	1098	1107	1116	1125	1134
TCT GGA CAT ACA AAT GCA GTT GCT ACA GTG AGA TGT CAG GCT GCA GAA CCA CAA					
S G H T N A V A T V R C Q A A E P Q					
1143	1152	1161	1170	1179	1188
ATT ATT ACA GGA AGC CAT GAT ACT ACA ATT CGA TTA TGG GAT CTG GTG GCT GGA					
I I T G S H D T T I R L W D L V A G					
1197	1206	1215	1224	1233	1242
AAA ACA AGA GTG ACA TTA ACA AAT CAC AAA AAA TCA GTT AGG GCT GTG GTT TTA					
K T R V T L T N H K K S V R A V V L					
1251	1260	1269	1278	1287	1296
CAT CCA AGA CAT TAC ACA TTT GCA TCT GGT TCT CCA GAT AAC ATA AAG CAG TGG					
H P R H Y T F A S G S P D N I K Q W					
1305	1314	1323	1332	1341	1350
AAA TTC CCT GAT GGA AGT TTC ATT CAA AAT CTT TCC GGT CAT AAT GCT ATT ATT					
K F P D G S F I Q N L S G H N A I I					

FIGURE 1D

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1359	1368	1377	1386	1395	1404
AAC ACA TTG ACG GTA AAT TCT GAT GGA GTG CTT GTA TCT GGA GCT GAC AAT GGC					
N T L T V N S D G V L V S G A D N G					
1413	1422	1431	1440	1449	1458
ACC ATG CAT CTT TGG GAC TGG AGA ACT GGC TAC AAT TTT CAG AGA GTT CAC GCA					
T M H L W D W R T G Y N F Q R V H A					
1467	1476	1485	1494	1503	1512
GCT GTG CAA CCT GGG TCT TTG GAC AGT GAA TCA GGA ATA TTT GCT TGT GCT TTT					
A V Q P G					
1521	1530	1539	1548	1557	1566
GAT CAG TCT GAA AGT CGA TTA CTA ACA GCT GAA GCT GAT AAA ACC ATT AAA GTA					
1575	1584	1593	1602	1611	1620
TAC AGA GAG GAT GAC ACA GCC ACA GAA GAA ACT CAT CCA GTC AGC TGG AAA CCA					
1629	1638	1647	1656	1665	1674
GAA ATT ATC AAG AGA AAG AGA TTT TAA TGA ATG TGG AAT TTT TTC TCT CTC TTT					
1683	1692	1701	1710	1719	1728
TTT TTT CTT TTT AAT TAA AAA AAA AGC TTG GCG TTC ATG AGG ATA TCC AGT					

FIGURE 1E

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1737	1746	1755	1764	1773	1782
CAT TTT GTG CTC TGG CTG GGA ATA TAA AGG AGA AAT TCA CTT GCT TCC AAT CCA					
1791	1800	1809	1818	1827	1836
TTG CTG CTT CAT ATT TTA CCA ATA AAC CTG TCC CCC TGT CCC CTA CCC CTG TGT					
1845	1854	1863	1872	1881	1890
TTT TAT TTC TAA AAC CAT TTT GGG GAT ACT AGG AAG TTG CAG ATA TCA AGT AAA					
1899	1908	1917	1926	1935	1944
TTG CAG GTT TAT TGA ACA TAA CTA TTC NTA GTG TAA TAT TTT GAC AGT CTT ATT					
1953	1962	1971	1980		
TGG AAA ACC CCC TTT TTT AAA AAA AAA TGT ATG GAA CCG					

FIGURE 1F

395 I K Q W K F P D G S F I O N - L S G H N A I I N T L T V N S D G V L V S G A D N 78563
367 T K K F S L P K G E F C H N M L S Q Q K T I I N A M A V N E D G V M V T G G D N GI 577733

434 G T M H L W D W R T G Y N F Q R V H A A V Q P G 78563
407 G S I W F W D W K S G H S F Q Q S E T I V Q P G S L E S E A G I Y A A C Y D N T GI 577733

457 78563
447 G S R L V T C E A D K T I K M W K E D E N A T P E T H P I N F K P P K E I R R F GI 577733

FIGURE 2B

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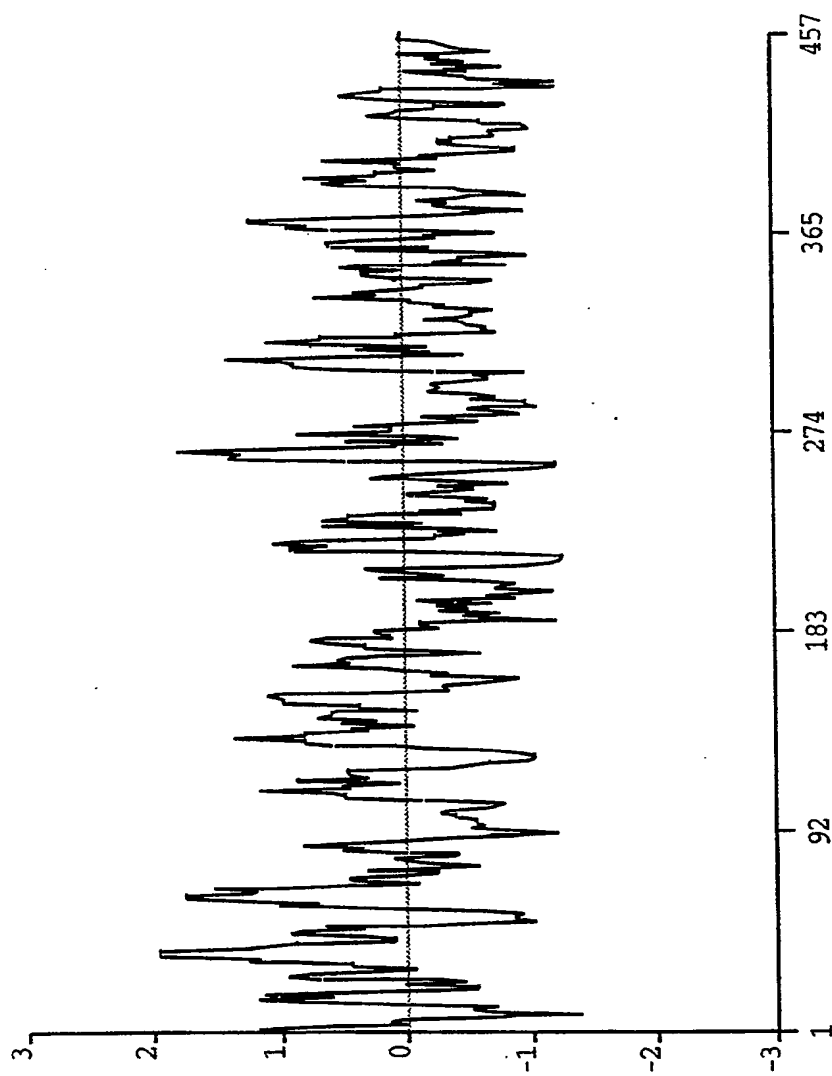


FIGURE 3A

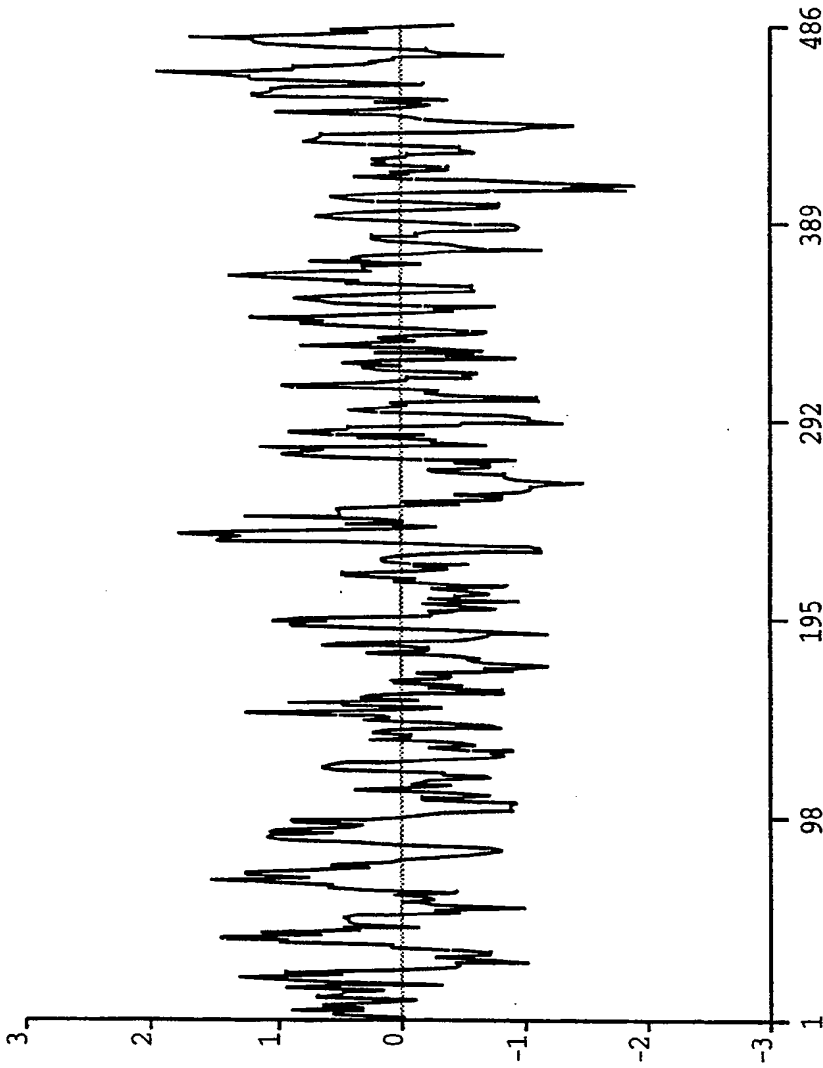


FIGURE 3B

<110> INCYTE PHARMACEUTICALS, INC.
 AU-YOUNG, Janice
 GUEGLER, Karl J.
 CORLEY, Neil C.

<120> HUMAN PRL1 PHOSPHATASE

<130> PF-0391 PCT

<140> To Be Assigned

<141> Herewith

<150> US 934,169

<151> 1997-09-19

<160> 3

<170> PERL Program

<210> 1

<211> 457

<212> PRT

<213> Homo sapiens

<220> -

<223> 78563

<400> 1

Met	Val	Glu	Glu	Val	Gln	Lys	His	Ser	Val	His	Thr	Leu	Val	Phe
1				5					10					15
Arg	Ser	Leu	Lys	Arg	Thr	His	Asp	Met	Phe	Val	Ala	Asp	Asn	Gly
				20					25					30
Lys	Pro	Val	Pro	Leu	Asp	Glu	Glu	Ser	His	Lys	Arg	Lys	Met	Ala
				35					40					45
Ile	Lys	Leu	Arg	Asn	Glu	Tyr	Gly	Pro	Val	Leu	His	Met	Pro	Thr
				50					55					60
Ser	Lys	Glu	Asn	Leu	Lys	Glu	Lys	Gly	Pro	Gln	Asn	Ala	Thr	Asp
				65					70					75
Ser	Tyr	Val	His	Lys	Gln	Tyr	Pro	Ala	Gly	Asn	Gly	Gln	Glu	Val
				80					85					90
Glu	Tyr	Phe	Val	Ala	Gly	Thr	His	Pro	Tyr	Pro	Pro	Gly	Pro	Gly
				95					100					105
Val	Ala	Leu	Thr	Ala	Asp	Thr	Lys	Ile	Gln	Arg	Met	Pro	Ser	Glu
				110					115					120
Ser	Ala	Ala	Gln	Ser	Leu	Ala	Val	Ala	Leu	Pro	Leu	Gln	Thr	Lys
				125					130					135
Ala	Asp	Ala	Asn	Arg	Thr	Ala	Pro	Ser	Gly	Ser	Glu	Tyr	Arg	His
				140					145					150
Pro	Gly	Ala	Ser	Asp	Arg	Pro	Gln	Pro	Thr	Ala	Met	Asn	Ser	Ile
				155					160					165
Val	Met	Glu	Thr	Gly	Asn	Thr	Lys	Asn	Ser	Ala	Leu	Met	Ala	Lys
				170					175					180
Lys	Ala	Pro	Thr	Met	Pro	Lys	Pro	Gln	Trp	His	Pro	Pro	Trp	Lys
				185					190					195
Leu	Tyr	Arg	Val	Ile	Ser	Gly	His	Leu	Gly	Trp	Val	Arg	Cys	Ile
				200					205					210
Ala	Val	Glu	Pro	Gly	Asn	Gln	Trp	Phe	Val	Thr	Gly	Ser	Ala	Asp
				215					220					225
Arg	Thr	Ile	Lys	Ile	Trp	Asp	Leu	Ala	Ser	Gly	Lys	Leu	Lys	Leu
				230					235					240
Ser	Leu	Thr	Gly	His	Ile	Ser	Thr	Val	Arg	Gly	Val	Ile	Val	Ser
				245					250					255
Thr	Arg	Ser	Pro	Tyr	Leu	Phe	Ser	Cys	Gly	Glu	Asp	Lys	Gln	Val
				260					265					270
Lys	Cys	Trp	Asp	Leu	Glu	Tyr	Asn	Lys	Val	Ile	Arg	His	Tyr	His

	275		280		285
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Asn Ala Val Ala	Thr Val Arg Cys Gln	Ala Ala Glu Pro Gln	Ile		
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Ile Thr Gly Ser	His Asp Thr Thr Ile	Arg Leu Trp Asp Leu	Val		
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Arg Ala Val Val	Leu His Pro Arg His	Tyr Thr Phe Ala Ser	Gly		
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Ile Gln Asn Leu	Ser Gly His Asn Ala	Ile Ile Asn Thr Leu	Thr		
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Val Asn Ser Asp	Gly Val Leu Val Ser	Gly Ala Asp Asn Gly	Thr		
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Lys Gln Ile Arg Leu Ser His Lys Met Lys Val Ala Phe Gly Gly
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Val Glu Pro Val Val Ser Gln Pro Pro Arg Gln Pro Asp Arg Ile
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Glu Gly Ser Lys Ser Thr Gln Lys Gly Ala Thr Glu Ser Ala Ile
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Trp Lys Asn Tyr Arg Val Ile Gln Gly His Leu Gly Trp Val Arg
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Gln	Val	Val	Thr	Gly	Ser	His	Asp	Thr	Thr	Ile	Lys	Phe	Trp	Asp	320	325	330
Leu	Arg	Tyr	Gly	Lys	Thr	Met	Ser	Thr	Leu	Thr	His	His	Lys	Lys	335	340	345
Ser	Val	Arg	Ala	Met	Thr	Leu	His	Pro	Lys	Glu	Asn	Ala	Phe	Ala	350	355	360
Ser	Ala	Ser	Ala	Asp	Asn	Thr	Lys	Lys	Phe	Ser	Leu	Pro	Lys	Gly	365	370	375
Glu	Phe	Cys	His	Asn	Met	Leu	Ser	Gln	Gln	Lys	Thr	Ile	Ile	Asn	380	385	390
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Gln	Gln	Ser	Glu	Thr	Ile	Val	Gln	Pro	Gly	Ser	Leu	Glu	Ser	Glu	425	430	435
Ala	Gly	Ile	Tyr	Ala	Ala	Cys	Tyr	Asp	Asn	Thr	Gly	Ser	Arg	Leu	440	445	450
Val	Thr	Cys	Glu	Ala	Asp	Lys	Thr	Ile	Lys	Met	Trp	Lys	Glu	Asp	455	460	465
Glu	Asn	Ala	Thr	Pro	Glu	Thr	His	Pro	Ile	Asn	Phe	Lys	Pro	Pro	470	475	480
Lys	Glu	Ile	Arg	Arg	Phe										485		

INTERNATIONAL SEARCH REPORT

Int. l. Application No
PCT/US 98/19805

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/55 C12N9/16 A61K38/46 C12Q1/68 C12N15/11
C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A61K C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL - EMBEST9 Entry HS351288, cc.No. N57351, 29 February 1996 HILLIER, L. ET AL.: "yw86d09.r1 Homo sapiens cDNA clone 259121 5' similar to PIR:S49820 S49820 PRL1 protein - Arabidopsis thaliana" XP002089394	3-8
Y	see the whole document ----- -/--	1,2,9-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 January 1999

Date of mailing of the international search report

22/01/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/19805

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE EMBL - R56U061 Entry ATPRL1RNA, Acc.No. X82825, 25 November 1994 NEMETH, K. ET AL.: "A.thaliana PRL1 mRNA." XP002089395 cited in the application see the whole document -& NEMETH, K. ET AL.: "Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in Arabidopsis. " GENES AND DEVELOPMENT, vol. 12, 1 October 1998, pages 3059-73, XP002089393</p>	1,2,9-19
A	<p>WO 97 06262 A (INCYTE PHARMA INC) 20 February 1997 see the whole document, particularly the claims</p>	12-17
A	<p>DIAMOND R H ET AL: "PRL-1, A UNIQUE NUCLEAR PROTEIN TYROSINE PHOSPHATASE, AFFECTS CELL GROWTH" MOLECULAR AND CELLULAR BIOLOGY, vol. 14, no. 6, June 1994, pages 3752-3762, XP000196604 see the whole document</p>	
P,X	<p>DATABASE EMBL - EMHUM1 Entry/Acc.No. Af044333, 5 February 1998 OKRESZ, L.: "Homo sapiens pleiotropic regulator 1 (PLRG1) mRNA, complete cds." XP002089396 see the whole document</p>	1-11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/19805

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 14, 15
because they relate to subject matter not required to be searched by this Authority, namely:
SEE FURTHER INFORMATION SHEET PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 16 and 17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Claims Nos.: 14,15

Claims 14 and 15, referring to (ant)agonists, could only be searched partially due to insufficient characterization of the compounds, and lack of support by the description.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 98/19805

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9706262 A	20-02-1997	AU 6688696 A	05-03-1997